

Electrophoretic patterns of heat-denatured collagen in the temperature range of 14–40°

In a previous publication¹ it was demonstrated that a "reconstituted" collagen yields a pattern on gel-electrophoresis at room temperature, which is different from the pattern obtained at 40°. We report here more systematic experiments on the starch-gel electrophoresis of heat-denatured collagen at lower temperatures, including the collagen \rightleftharpoons gelatin phase-transition range².

The collagen samples were obtained from the tendon fibres of rat tail by extraction with 20 volumes of 3 % (v/v) acetic acid in the cold for 24 h. The native material had been extracted exhaustively beforehand with 0.4 M NaCl, buffered at pH 7.3 with 0.067 M phosphate and the supernatants had been discarded. The collagen in the acetic acid extract was precipitated by dialysis against 0.067 M phosphate buffer (pH 7.3) and distilled water. The precipitate was lyophilized, dissolved in 3 % acetic acid and dialyzed against acetate buffer (pH 4.5, *I* 0.022), which was also used in the preparation of the starch gel for the electrophoresis and in the electrode compartments. The final concentration of collagen was adjusted to 1 %. Before each run the sample (40 μ l) was denatured at 40° for 15 min, imbibed in a paper strip and inserted into a slot in the starch gel, where it was allowed to equilibrate to the desired temperature for

30 min before the current was passed. The equipment provided for an efficient stabilization of the temperature in the gel by a continuous circulation of water from a temperature-controlled vessel through cooling chambers on both sides of the gel. The gel contained 13 g of hydrolyzed starch (Connaught Research Laboratories Ltd., Toronto) in 100 ml. The run was carried out vertically with a voltage of 200 V, thus giving a current of 12–15 mA in the gel sheet of 6 mm \times 58 mm \times 143 mm. The running time was 20 h (except at 30° when the time was 14 h).

The results are shown in Fig. 1. At 30° the α -fractions (large aggregates) are retained on the starting line; at 26° the α_2 -, α_1 - and β_1 -units migrate, at 22° the α_2 - and α_1 -units migrate and at 18° the α_2 -unit alone migrates. The non-denatured soluble collagen does not move under the present conditions. For the identification of the bands a separate run was carried out at 22°, the two fractions which were produced were cut out separately and further fractionated at 40°, and it was thus ascertained that the two fractions are the same as designated α_1 and α_2 in the regular pattern obtained at 40°. At 14° an essentially similar pattern was obtained as at 18°.

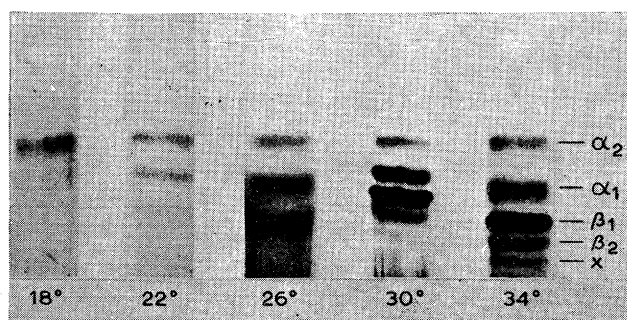


Fig. 1. Starch-gel electrophoretic pattern of the same denatured collagen sample from the tendon of rat tail, carried out at indicated temperatures.

As an explanation for these findings we suggest that the refolding of the various subunits of heat-denatured collagen does not happen at the same temperature. It seems reasonable that the multi-chain fragments (α and β) fold easier than the α -units. The "renaturation" on cooling of gelatin is augmented by the presence of β - and γ -components³. The electrophoretic behaviour at various temperatures offers also an additional possibility for a preparative separation of the components by a "temperature-programmed" electrophoresis.

Support by institutional grants from U.S. Department of Agriculture, Foreign Research and Technical Programs Division, and from the Sigrid Jusélius Foundation is gratefully acknowledged.

Department of Medical Chemistry,
University of Turku, Turku (Finland)

T. HOLLMÉN
E. KULONEN

¹ V. NÄNTÖ, J. MAATELA AND E. KULONEN, *Acta Chem. Scand.*, 17 (1963) 1604.

² P. H. V. HIPPEL AND K.-Y. WONG, *Biochemistry*, 2 (1963) 1387.

³ K. ALTGELT, A. J. HODGE AND F. O. SCHMITT, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 1914.

Received July 10th, 1964